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Chronic Exposure of Ruminal Fluid Cultures to Treatments That Inhibit Methanogenesis

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Summary

Methanogenesis in ruminal cultures was inhibited by Yucca shidigera, 2-bromoethanesulfonate, and a nitrofuranyl para-aminobenzoic acid derivative. Only the nitrofuranyl para-aminobenzoic acid derivative remained effective beyond 10 days of culture indicating that this may be an effective treatment for chronically inhibiting methane production in cattle.

Introduction

Sustained inhibition of ruminal methane production would result in a retention of feed gross energy and may improve feed efficiency by up to one-third. An historical theme associated with treatments that inhibit methanogenesis is the ability of ruminal methanogens to adapt to the effects of such treatments and become resistant following chronic exposure. For practical purposes an inhibitor of ruminal methane production must: 1) be effective at small enough concentrations that the treatment can be included as a dietary supplement and 2) no adaptation to this treatment should occur. A nitrofuranyl para-aminobenzoic acid derivative (NFP) meets the former requirement and was previously reported (2006 Nebraska Beef Report, pp. 83-84; compound C33) to inhibit in vitro methane production by > 98% at a concentration of 1.0 mM. Unpublished data from our laboratory also indicate an extract from *Yucca shidigera* (Yucca), which is commercially available and approved for feeding, inhibits methane production by > 65% when included in cultures

of ruminal fluid at a concentration of 25 μ L/mL. 2-bromoethanesulfonate (BES) is a potent inhibitor of methanogenesis, but ruminal methanogens quickly acquire resistance to the effects of this compound. The objective of our work was to determine the extent to which ruminal cultures acquire resistance following chronic exposure to NFP, Yucca, and BES, a positive control for resistance development.

Procedure

Ruminal fluid from a fistulated heifer receiving a mixed forage and concentrate diet was used to inoculate chronic cultures (n = 8) exposed to no treatment (control) or a low concentration of NFP, Yucca, or BES (100 μ M, 2.5 μ L/mL, and 10 μ M, respectively). In addition to the ruminal fluid inoculum chronic cultures contained McDougall's buffer, distilled H₂O, cellobiose, trypticase, resazurin, a micro mineral solution, and Na₂S. The fermentation media were gassed with CO₂ to create oxygen-free media and then added to a 120 mL glass vial, which contained the respective treatments, as oxygen free gas (H₂/CO₂, 80:20) was projected into each vial. The vials were sealed, pressurized to 100 kPa (1 atmosphere), and allowed to incubate in a water bath (102 F) for 90 d. Every 2 d 50% of the medium from each vial was replaced with fresh culture medium, which contained the same components as the original medium with the exception that clarified ruminal fluid was substituted for fresh ruminal fluid. The fresh medium contained an identical concentration of each treatment as the medium it replaced allowing for the concentration of inhibitor to remain constant for the duration of the experiment.

On day 0, ruminal fluid from the same source used to inoculate chronic cultures was used to inoculate

acute cultures (n = 4/treatment) that received no treatment (control), NFP (100 μ M or 1,000 μ M), Yucca (2.5 μ L/mL or 25 μ L/mL) or BES (10 μ M or 100 μ M). On days 2, 10, 22, 32, 40, 60, and 90, media removed from chronic cultures were also used to inoculate acute cultures treated in duplicate with either 0 or 10X the same inhibitor as used for creating the chronic culture inoculum (n = 24/d). Control-inoculated acute cultures were also treated in duplicate as controls and with these 10X doses of NFP, BES, and Yucca (n = 16/d). Excluding inocula source, the acute cultures contained proportionally identical ingredients compared to the chronic cultures and were prepared identically as 4 mL of fermentation media in 10 mL glass vials incubated in a water bath (102°F) for 18 hours. Following incubation, pressure in the headspace of the vials was measured. Methane concentration was determined by gas chromatography using a silica packed column and thermal conductivity detector.

Data were analyzed utilizing the MIXED procedure of SAS. The model for methane produced by day 0 cultures included the fixed effect of treatment. The model for methane produced by days 2, 10, 22, 32, 40, 60 and 90 acute cultures included the fixed effects of previous exposure to a 1X dose of an inhibitor, treatment with a 10X dose of an inhibitor, the random effect of day that the media were removed from continuous cultures and used to inoculate an acute culture, and all appropriate two-way and three-way interactions. Because the same medium was sampled across days a repeated measures covariance structure was used.

Results

Day 0 Acute Cultures

Acute cultures inoculated with ruminal fluid from the same source that

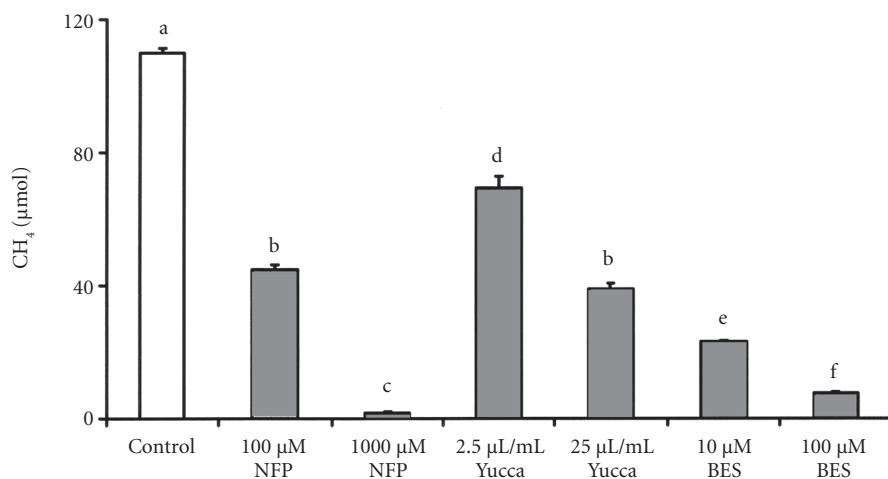


Figure 1. Methane produced by acute cultures inoculated with ruminal fluid from the same source as the ruminal fluid used to inoculate chronic cultures and treated with a high and low concentration of NFP, Yucca, or BES.
[a,b,c,d,e,f]Methane production differs ($P < 0.05$).

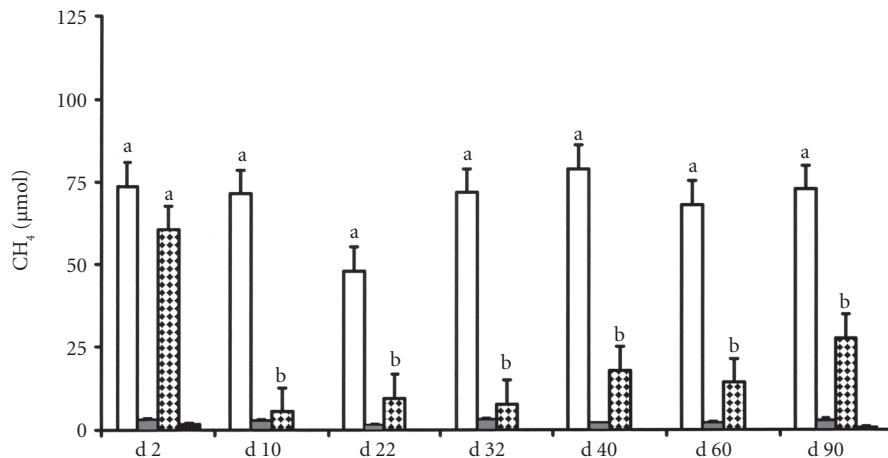


Figure 2. Methane produced by acute cultures inoculated with media from chronic control cultures and receiving no treatment (open bars) or a 10X concentration of NFP (gray bars), or inoculated with media from cultures chronically treated with NFP and receiving no treatment (checkered bars) or a 10X concentration of NFP (black bars).
[a,b]Methane production within day differs ($P < 0.05$).

was used to inoculate chronic cultures produced less ($P < 0.05$) methane when treated with a low dose of NFP, Yucca, and BES (100 μM, 2.5 μL/mL, and 10 μM, respectively; Figure 1) compared to control cultures. Increasing the concentration of every inhibitor 10X resulted in a further reduction ($P < 0.05$) in the amount of methane produced in vitro. Based on previous findings the low dose of each inhibitor, which was used to continuously treat the chronic cultures, was expected to significantly reduce methane production without completely

inhibiting in vitro methanogenesis. The results from this acute culture confirmed the concentrations of each inhibitor used were having the desired effect and also indicated the higher dose exacerbates this effect.

NFP

The ability of an inoculum to produce methane following chronic exposure to NFP is presented in Figure 2. The amount of methane produced by all acute cultures treated with a 10X concentration of NFP was

less than the sensitivity of our gas chromatograph and these data were excluded from the analysis. There was no difference in the amount of methane produced by cultures inoculated with a medium chronically exposed to NFP and control media on day 2. However, by day 10 acute cultures inoculated with media chronically exposed to NFP produced less methane ($P < 0.05$) than did acute cultures inoculated with control media and this relationship persisted for the remainder of the experiment. Exposure to NFP and time did interact to affect amount of methane produced by acute cultures ($P < 0.05$), but from day 10 to day 90 there was no difference in the amount of methane produced by acute cultures inoculated with media chronically exposed to NFP. These results indicate chronic cultures did not acquire resistance to effects of NFP and also indicate NFP may be efficacious for chronically inhibiting methanogenesis in cattle.

Yucca

The ability of an inoculum to produce methane following chronic exposure to Yucca is presented in Figure 3. Treatment of acute cultures with a 10X concentration of Yucca inoculated with media from control chronic cultures produced less methane ($P < 0.05$) than did control acute cultures receiving the same inoculum on days 10 through 90. With the exception of day 10, treatment of acute cultures with a 10X concentration of Yucca inoculated with media chronically exposed to Yucca resulted in a similar or greater ($P < 0.05$) amount of methane produced. Collectively, these data indicate Yucca treatment did maintain its capacity to inhibit methanogenesis for the duration of the experiment, but chronic treatment of cultures with Yucca diminishes the effectiveness of this treatment. Our interpretation is continuous Yucca treatment would not be an effective strategy for chronically inhibiting methane production.

(Continued on next page)

The ability of an inoculum to produce methane following chronic exposure to BES is presented in Figure 4. The amount of methane produced by acute cultures treated with a 10X concentration of BES and inoculated with media from chronic control cultures was less than the sensitivity of our gas chromatograph and these data were excluded from the analysis. Acute cultures receiving no treatment and inoculated with media from chronic cultures exposed to BES produced less methane ($P<0.05$) than did control cultures on day 2. By d 10 there was no difference in the amount of methane produced by these two groups of acute cultures and this relationship persisted for the duration of the experiment. These data indicate following chronic exposure to BES the cultures became resistant to the effects of this treatment. Treatment of acute cultures inoculated with chronically exposed media with a 10X concentration of BES resulted in a reduction ($P<0.05$) in the amount of methane produced on all days. Therefore, we cannot exclude the possibility that ruminal methanogenesis can be chronically inhibited by increasing the concentration of BES over time.

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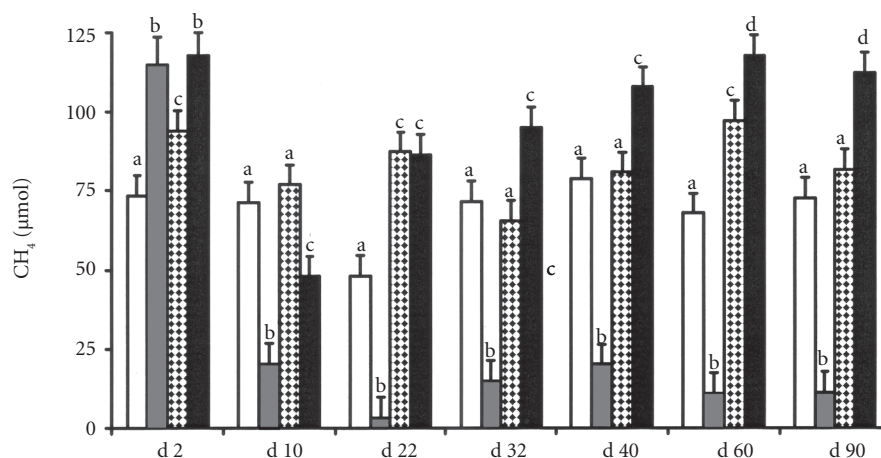


Figure 3. Methane produced by acute cultures inoculated with media from chronic control cultures and receiving no treatment (open bars) or a 10X concentration of Yucca (gray bars), or inoculated with media from cultures chronically treated with Yucca and receiving no treatment (checkered bars) or a 10X concentration of Yucca (black bars).
[a,b,c,d]Methane production within day differs ($P<0.05$).

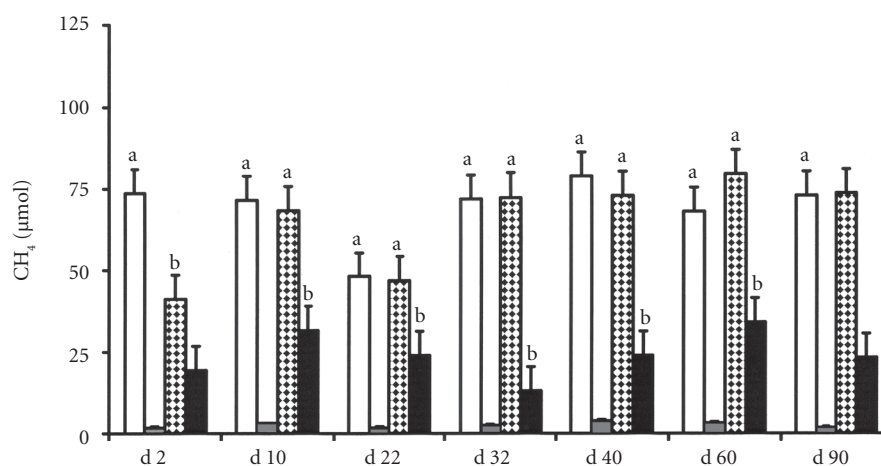


Figure 4. Methane produced by acute cultures inoculated with media from chronic control cultures and receiving no treatment (open bars) or a 10X concentration of BES (gray bars), or inoculated with media from cultures chronically treated with BES and receiving no treatment (checkered bars) or a 10X concentration of BES (black bars).
[a,b,c]Methane production within days differs ($P<0.05$).